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(54) Title: MR IMAGING COMPOSITIONS, BASED ON POLY(ALKYLENEOXIDE)					
(57) Abstract					
This invention provides compositions useful in MR imaging comprising a polymer comprising units comprising the residue of a chelating agent linked to a poly(alkyleneoxide) moiety, the polymer having a paramagnetic metal ion associated therewith.					
$  \begin{array}{c}  \text{TSO}(\text{CH}_2\text{CH}_2\text{O})_n\text{CH}_2\text{CH}_2\text{OTs} \xrightarrow[\text{TsCl}]{\text{R}^2\text{NH}_2} \text{R}^2\text{NH}(\text{CH}_2\text{CH}_2\text{O})_n\text{CH}_2\text{CH}_2\text{NHR}^2 \\  \text{HO}(\text{CH}_2\text{CH}_2\text{O})_n\text{CH}_2\text{CH}_2\text{OH} \\  \text{(PEO)}  \end{array}  \xrightarrow{\text{DTPA-dianhydride}}  \begin{array}{c}  \text{N}(\text{CH}_2\text{CH}_2\text{O})_n\text{CH}_2\text{CH}_2\text{N}(\text{Ts})\text{C}(=\text{O})\text{CH}_2\text{CO}_2\text{H} \\  \text{3}  \end{array}  \xrightarrow{\text{GdCl}_3}  \begin{array}{c}  \text{N}(\text{CH}_2\text{CH}_2\text{O})_n\text{CH}_2\text{CH}_2\text{N}(\text{Gd}^{3+})\text{C}(=\text{O})\text{CH}_2\text{CO}_2\text{H} \\  \text{4}  \end{array}  $ $  \begin{array}{c}  \text{B4A} \\  \text{N}(\text{CH}_2\text{CH}_2\text{O})_n\text{CH}_2\text{CH}_2\text{N}(\text{Ts})\text{C}(=\text{O})\text{CH}_2\text{CO}_2\text{H} \\  \text{P4A} \\  \text{N}(\text{CH}_2\text{CH}_2\text{O})_n\text{CH}_2\text{CH}_2\text{N}(\text{Gd}^{3+})\text{C}(=\text{O})\text{CH}_2\text{CO}_2\text{H}  \end{array}  $					

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## MR IMAGING COMPOSITIONS, BASED ON POLY(ALKYLENEOXIDE)

CROSS REFERENCE TO RELATED APPLICATIONS

5 This application is a continuation-in-part of U.S. Patent Application Ser. No. 07/960,746 filed October 14, 1992.

FIELD OF INVENTION

10 This invention relates to chelating polymers useful as magnetic resonance (MR) contrast agents for use in MR imaging compositions and methods.

BACKGROUND OF THE INVENTION

15 Magnetic resonance (MR) is widely used for obtaining spatial images of human subjects for clinical diagnosis. A review of this technology and clinical applications is provided by D.P. Swanson et al, in

20 Pharmaceuticals in Medical Imaging, 1990, Macmillan Publishing Company, pages 645-681.

25 MR images are a composite of the effects of a number of parameters which are analyzed and combined by computer. Choice of the appropriate instrument parameters, such as radio frequency (Rf), pulsing and timing can be utilized to enhance or attenuate the signals of any of the image-producing parameters thereby improving image quality and providing better anatomical and functional information. In many cases, MR imaging has proven to be a valuable

30 diagnostic tool, as normal and diseased tissue, by virtue of their possessing different parameter values, can be differentiated in the image.

35 In MR imaging, the in vivo image of an organ or tissue is obtained by placing the body of a subject in a strong external magnetic field, pulsing with radio frequency energy, and observing the effect of the pulses on the

magnetic properties of the protons contained in and surrounding the organ or tissue. A number of parameters can be measured. The proton relaxation times,  $T_1$  and  $T_2$ , are of primary importance.  $T_1$ , also called the spin-lattice or 5 longitudinal relaxation time, and  $T_2$ , also called the spin-spin or transverse relaxation time, depend on the chemical and physical environment of the organ or tissue water and are measured using Rf pulsing techniques. This information is analyzed as a function of spatial location by computer which 10 uses the information to generate an image.

Often the image produced lacks appropriate contrast, e.g., between normal and diseased tissue, reducing diagnostic effectiveness. To overcome this drawback, contrast agents have been used. Contrast agents are 15 substances which exert an effect on the MR parameters of various chemical species around them. Theoretically, a contrast agent, if taken up preferentially by a certain portion of an organ or a certain type of tissue, e.g., diseased tissue, can provide contrast enhancement in the 20 resultant images.

Inasmuch as MR images are strongly affected by variations in the  $T_1$  and  $T_2$  parameters, it is desirable to have a contrast agent which effects either or both parameters. Research has focused predominantly on two 25 classes of magnetically active materials, i.e., paramagnetic materials, which act primarily to decrease  $T_1$ , and superparamagnetic materials, which act primarily to decrease  $T_2$ .

Paramagnetism occurs in materials that contain 30 unpaired electrons. Paramagnetic materials are characterized by a weak magnetic susceptibility (response to an applied magnetic field). Paramagnetic materials become weakly magnetic in the presence of a magnetic field and rapidly lose

such activity, i.e., demagnetize, once the external field has been removed. It has long been recognized that the addition of paramagnetic solutes to water causes a decrease in the  $T_1$  parameter.

5        Paramagnetic materials, for example, Gd containing materials, have been used as MR contrast agents primarily because of their effect on  $T_1$ . Gd has the largest number of unpaired electron (seven) in its 4f orbitals and exhibits the greatest longitudinal relaxivity of any element.

10      A major concern with the use of contrast agents for MR imaging is that many paramagnetic materials exert toxic effects on biological systems making them inappropriate for in vivo use. For example, the free form of Gd is quite toxic. To make it suitable for in vivo use, researchers have 15 chelated it with diethylenetriaminepentaacetic acid (DTPA). A formulation of this material that has undergone extensive clinical testing consists of Gd-DTPA neutralized with two equivalents of N-methyl-D-glucamine (meglumine). This agent has been successful in enhancing human brain and renal 20 tumors.

Despite its satisfactory relaxivity and safety, this formulation has several disadvantages. For example, due to its low molecular weight, Gd-DTPA dimeglumine is cleared very rapidly from the blood stream and tissue lesions 25 (tumors). This limits the imaging window, the number of optimal images that can be taken after each injection, and increases the agents required dose and relative toxicity. In addition, the biodistribution of Gd-DTPA is suboptimal for imaging body tumors and infections due to its small molecular 30 size.

Several approaches have been taken in attempts to overcome these disadvantages. For example, Gd and Gd-chelates have been chemically conjugated to macromolecular

proteins such as albumin, polylysines and immunoglobulins. Drawbacks of conjugating DTPA to protein carriers for use in MR image enhancement include inappropriate biodistribution and toxicity. In addition, proteins provide a defined 5 platform not subject to wide synthetic variation. Additionally, thermal sterilization of protein conjugates tends to be problematic, especially in the case of albumin conjugates.

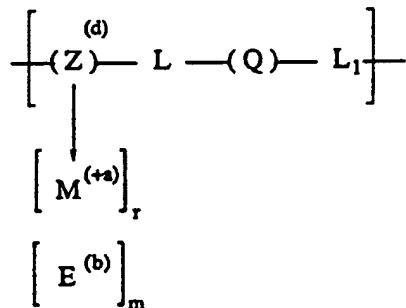
Thus, it is readily apparent that it would be 10 highly desirable to provide easily produced MR contrast agents which contain relatively large amounts of metal per molecule, i.e., are of high substitution ratios, yet are of a molecular weight enabling them to be circulated within the blood pool for extended periods of time, and/or which exhibit 15 improved biodistribution for imaging blood vessels, body tumors and other tissues.

#### SUMMARY OF THE INVENTION

20 We have discovered that reactive poly(alkylene oxides) can be contacted with chelating agents or precursors thereof containing reactive functionality to form metallizable polymers which when associated with paramagnetic metal ions provide polymeric chelates of 25 extraordinary utility as contrast agents for MR imaging.

More particularly, in accordance with this invention, there is provided a polymer, suitable for use as a contrast agent for MR imaging, containing units comprising the residue of a chelating agent linked to a poly(alkylene oxide) moiety, such polymer having a paramagnetic metal ion 30 associated therewith. The polymer preferably comprises units having the structure

I.



wherein:

5        Z is the residue of a chelating agent;  
       Q is a poly(alkylene oxide) moiety;  
       L and L<sub>1</sub> independently represent a chemical bond or a  
       linking group;  
       M<sup>(+a)</sup> is one or more paramagnetic metal ions having a  
 10      total charge of +a;  
       E<sup>(b)</sup> is one or more counterions having a total charge  
       of b;  
       m is 0 or 1;  
       r is 0 or 1;  
 15      d is the total charge on the linked residue of the  
       chelating group; and  
       a = d+b.

This invention further provides a method of  
 performing an MR diagnostic procedure in a body comprising  
 20      administering to the body a contrast enhancing amount of the  
       polymer described above, and then exposing the body to an MR  
       measurement step to image at least a portion of the body.

It is a particularly advantageous feature that the  
 paramagnetic polymeric chelates of this invention provide  
 25      effective MR contrast enhancement of the blood pool within  
       the vascular system for remarkably long periods of time.

It is an advantageous feature of this invention that polymeric compounds are provided having a specificity to accumulation in different tissues, for example, in tumors and the liver.

5 It is another advantageous feature of this invention that polymeric chelates suitable for MR imaging are provided containing relatively large amounts of metal that are potentially more stable in vivo and less immunoreactive than protein-chelate-metal complexes.

10 Yet another advantageous feature of this invention is that the molecular weight of the above-described polymers can be synthetically tailored to produce an agent of desired composition, molecular weight and size.

15 Still other advantageous features of this invention will become readily apparent upon reference to the following description of preferred embodiments.

#### BRIEF DESCRIPTION OF THE DRAWINGS

20 Figure 1 is a plot of signal enhancement over time for various compositions of this invention and a prior art control in a Rabbit V-2 Tumor Model.

25 Figures 2 and 3 are MR images of the abdominal area of a rabbit before and after administration of a contrast enhancing composition in accordance with this invention.

#### DESCRIPTION OF PREFERRED EMBODIMENTS

30 While the polymer of this invention is described herein primarily in connection with its preferred utilities, i.e., as a contrast agent for use in MR imaging compositions and methods, it also finds utility in other applications and fields, e.g., as a therapeutic agent and as an additive for

paints, coatings and adhesives and as an antistatic agent for use in photographic and magnetic recording elements.

The polymer useful in the practice of the invention contains units comprising the residue of a chelating agent 5 linked to a poly(alkylene oxide) moiety in the backbone of the polymer chain. The polymer comprises from 2 to 1000 or more, preferably 3 to 1000, of the above described units. In preferred embodiments, the above-described units are recurring units.

10 In formula I above, Q represents a linear or branched poly(alkylene oxide) moiety. Exemplary poly(alkylene oxide) moieties include poly(ethylene oxides), poly(propylene oxides) and poly(butylene oxides). Preferred poly(alkylene oxides) include poly(ethylene oxides) (PEO), 15 poly(propylene oxides) (PPO) and random and block copolymers of PEO and PPO. PEO containing polymers are particularly preferred when it is desired for the final polymer to possess solubility in water. It is also contemplated that the poly(alkylene oxide) moiety can comprise glycerol 20 poly(alkylene oxide) triethers and linear and block copolymers of alkylene oxides with compatible comonomers such as poly(ethyleneimine-co-ethylene oxide) and grafted block copolymers such as poly(methyl vinyl ether-co-ethylene oxide). For MR imaging applications, preferred poly(alkylene 25 oxide) moieties have an average molecular weight in the range from about 100-20,000, more preferably 250-10,000 daltons. These moieties can be derived from poly(alkylene oxide) moieties which are commercially available in the corresponding diol form and/or can be prepared by techniques 30 well known to those skilled in the art. A particularly preferred class of PEO moieties derived from PEGs can be represented by the structure  $-(CH_2CH_2O)_mCH_2CH_2-$  wherein m is 1 to 5,000, preferably 1 to 2500, and more preferably 1 to 500.

In a particularly preferred embodiment, the polymer of this invention comprises PEO moieties represented by the structure  $\text{-}(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2\text{-}$  wherein  $m$  is from 1 to about 6. This class of polymers exhibits a particularly high 5 gadolinium content and doses well. In addition, the PEO moieties wherein  $m=2$  or 3 can be derived from PEGs which are commercially available. In highly preferred embodiments,  $Q$  represents

10  $\text{-}(\text{CH}_2\text{CH}_2\text{O})_3\text{CH}_2\text{CH}_2\text{-}$ ;  
 $\text{-}(\text{CH}_2\text{CH}_2\text{O})_2\text{CH}_2\text{CH}_2\text{-}$ ; or  
 $\text{-CH}_2\text{-}(\text{CH}_2\text{CH}_2\text{O})_3\text{CH}_2\text{CH}_2\text{CH}_2\text{-}$ .

The polymer of the invention can comprise the residue of one or more of a wide variety of chelating agents. As is well known, a chelating agent is a compound containing 15 donor atoms that can combine by coordinate bonding with a metal atom to form a cyclic structure called a chelation complex or chelate. This class of compounds is described in the Kirk-Othmer Encyclopedia of Chemical Technology, Vol. 5, 339-368.

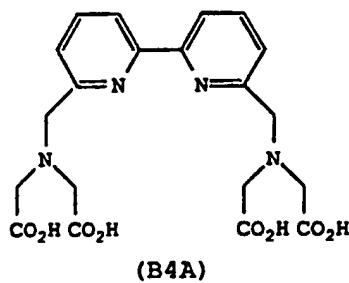
20 The residues of suitable chelating agents can be selected from polyphosphates, such as sodium tripolyphosphate and hexametaphosphoric acid;

aminocarboxylic acids, such as ethylenediaminetetraacetic acid, N-(2-hydroxyethyl)ethylenediaminetriacetic acid, nitrilotriacetic acid, N,N-di(2-hydroxyethyl)glycine, ethylenebis(hydroxyphenylglycine) and diethylenetriamine pentacetic acid;

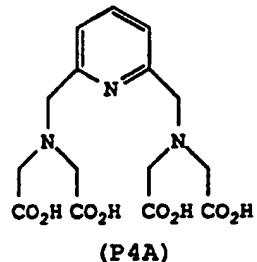
1,3-diketones, such as acetylacetone, trifluoroacetylacetone, and thenoyltrifluoroacetone; hydroxycarboxylic acids, such as tartaric acid, citric acid, gluconic acid, and 5-sulfosalicylic acid; polyamines, such as ethylenediamine, diethylenetriamine,

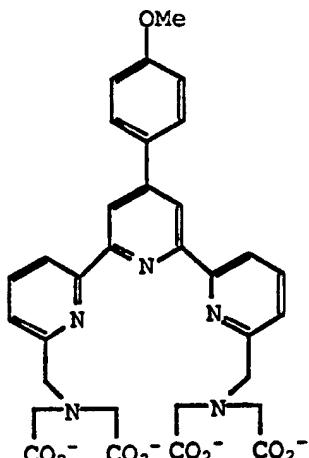
triethylenetetramine, and triaminotriethylamine; aminoalcohols, such as triethanolamine and N-(2-hydroxyethyl)ethylenediamine; aromatic heterocyclic bases, such as 2,2'-dipyridyl, 5 2,2'-diimidazole, dipicoline amine and 1,10-phenanthroline; phenols, such as salicylaldehyde, disulfopyrocatechol, and chromotropic acid; aminophenols, such as 8-hydroxyquinoline and oxinesulfonic acid; 10 oximes, such as dimethylglyoxime and salicylaldoxime; peptides containing proximal chelating functionality such as polycysteine, polyhistidine, polyaspartic acid, polyglutamic acid, or combinations of such amino acids; Schiff bases, such as disalicylaldehyde 1,2- 15 propylenediamine; tetrapyrroles, such as tetraphenylporphin and phthalocyanine; sulfur compounds, such as toluenedithiol, meso-2,3-dimercaptosuccinic acid, dimercaptopropanol, thioglycolic 20 acid, potassium ethyl xanthate, sodium diethyldithiocarbamate, dithizone, diethyl dithiophosphoric acid, and thiourea; synthetic macrocyclic compounds, such as dibenzo[18]crown-6,  $(CH_3)_6[14]4,11$ -dieneN<sub>4</sub>, and (2.2.2- 25 cryptate); and phosphonic acids, such as nitrilotrimethylenephosphonic acid, ethylenediaminetetra(methylenephosphonic acid), and hydroxyethylidenediphosphonic acid, or combinations of two or more of the above agents. 30 Preferred residues of chelating agents contain polycarboxylic acid groups and include: ethylenediamine-N, N, N',N'-tetraacetic acid (EDTA); N,N,N',N",N"-diethylenetriaminepentaacetic acid (DTPA); 1,4,7,10-

tetraazacyclododecane-N,N',N",N'''-tetraacetic acid (DOTA);  
1,4,7,10-tetraazacyclododecane-N,N',N"-triacetic acid (DO3A);  
1-oxa-4,7,10-triazacyclododecane-N,N',N"-triacetic acid  
(OTTA); trans(1,2)-cyclohexanodiethylenetriamine pentaacetic  
5 acid (CDTPA)

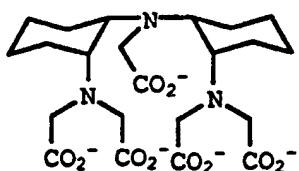


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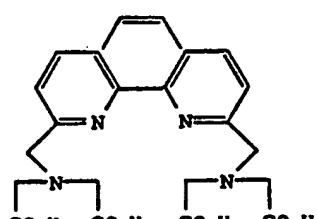


(TMT);



(DCDTPA); and

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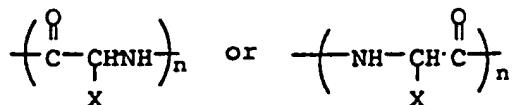


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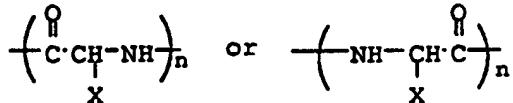
Other suitable chelating groups are described in  
 10 PCT/US91/08253, the disclosure of which is hereby  
 incorporated by reference. In formula I above, Z is the  
 residue of one or more chelating agents. If Z is the residue

of multiple chelating units, such units can be linked together by a linking group such as described below.

The residue of the chelating agent is linked to the poly(alkylene oxide) moiety through a chemical bond or 5 a linking group, i.e., L and L<sub>1</sub> in formula I above. Preferred linking groups include nitrogen atoms in groups such as amino, imido, nitrilo and imino groups; alkylene, preferably containing from 1 to 18 carbon atoms such as 10 methylene, ethylene, propylene, butylene and hexylene, such alkylene optionally being interrupted by 1 or more heteroatoms such as oxygen, nitrogen and sulfur or heteroatom-containing groups; carbonyl; sulfonyl; 15 sulfinyl; ether; thioether; ester, i.e., carbonyloxy and oxycarbonyl; thioester, i.e., carbonylthio and thiocarbonyl; 20 amide, i.e., iminocarbonyl and carbonylimino; thioamide, i.e., iminothiocarbonyl and thiocarbonylimino; thio; dithio; phosphate; 25 phosphonate; urelene; thiourelene; urethane, i.e., iminocarbonyloxy; thiourethane, i.e., iminothiocarbonylthio; 30 an amino acid linkage, i.e., a



group wherein n=1 and X is H, alkyl, containing from 1 to 18, preferably 1 to 6 carbon atoms, such as methyl, ethyl and 5 propyl, such alkyl optionally being interrupted by 1 or more heteroatoms such as oxygen, nitrogen and sulfur, substituted or unsubstituted aryl, containing from 6 to 18, preferably 6 to 10 carbon atoms such as phenyl, hydroxyiodophenyl, hydroxyphenyl, fluorophenyl and naphthyl, aralkyl, preferably 10 containing from 7 to 12 carbon atoms, such as benzyl, heterocyclyl, preferably containing from 5 to 7 nuclear carbon and one or more heteroatoms such as S, N, P or O, examples of preferred heterocyclyl groups being pyridyl, quinolyl, imidazolyl and thienyl; heterocyclylalkyl, the 15 heterocyclyl and alkyl portions of which preferably are described above; or a peptide linkage, i.e., a



group wherein n>1 and each X independently is represented 20 by a group as described for X above. Two or more linking groups can be used, such as, for example, alkyleneimino and iminoalkylene. It is contemplated that other linking groups may be suitable for use herein, such as linking groups commonly used in protein heterobifunctional and 25 homobifunctional conjugation and crosslinking chemistry. Especially preferred linking groups are unsubstituted or substituted imino groups which when linked to the carbonyl in the residue of a chelating agent form an amide.

The linking groups can contain various substituents

which do not interfere with the polymerization reaction. The linking groups can also contain substituents which can interfere with the polymerization reaction, but which during the polymerization reaction, are prevented from so doing with

5 suitable protecting groups commonly known in the art and which substituents are regenerated after the polymerization by suitable deprotection. The linking groups can also contain substituents that are introduced after the polymerization. For example, the linking group can be

10 substituted with substituents such as halogen, such as F, Cl, Br or I; an ester group; an amide group; alkyl, preferably containing from 1 to about 18, more preferably, 1 to 4 carbon atoms such as methyl, ethyl, propyl, i-propyl, butyl, and the like; substituted or unsubstituted aryl, preferably

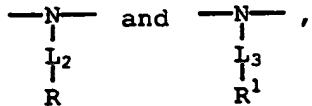
15 containing from 6 to about 20, more preferably 6 to 10 carbon atoms such as phenyl, naphthyl, hydroxyphenyl, iodophenyl, hydroxyiodophenyl, fluorophenyl and methoxyphenyl; substituted or unsubstituted aralkyl, preferably containing from 7 to about 12 carbon atoms, such as benzyl and

20 phenylethyl; alkoxy, the alkyl portion of which preferably contains from 1 to 18 carbon atoms as described for alkyl above; alkoxyaralkyl, such as ethoxybenzyl; substituted or unsubstituted heterocyclyl, preferably containing from 5 to 7 nuclear carbon and heteroatoms such as S, N, P or O, examples

25 of preferred heterocyclyl groups being pyridyl, quinolyl, imidazolyl and thienyl; a carboxyl group; a carboxyalkyl group, the alkyl portion of which preferably contains from 1 to 8 carbon atoms; the residue of a chelating group, preferably such as described for Z above; or a poly(alkylene

30 oxide) moiety, preferably such as described for Q above.

In a preferred embodiment, L and L<sub>1</sub>, represent



wherein  $\text{L}_2$  and  $\text{L}_3$  independently represent a chemical bond or a linking group such as described above, and  $\text{R}$  and  $\text{R}^1$  independently represent H; or a substituent attached to the 5 linking group such as described above.

$\text{E}$  can be one or more counterions. For example,  $\text{E}$  can be one or more anions, such as a halide, such as chloride and iodide; sulfate; phosphate; nitrate; and acetate.  $\text{E}$  can be one or more cations such as  $\text{Na}^+$ ,  $\text{K}^+$ , meglumine, and the 10 like. For in vivo applications, nontoxic physiologically tolerable anions are, of course, desirable.

For MR imaging applications,  $\text{M}^{(+a)}$  preferably represents a paramagnetic metal ion such as an ion of metals of atomic number 21 to 29, 42, 44 and 57 to 71, especially 57 15 to 71. Ions of the following metals are preferred: Cr, V, Mn, Fe, Co, Ni, Cu, Ce, Pr, Nd, Pm, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, and Yb. Especially preferred are  $\text{Cr}^{+3}$ ,  $\text{Cr}^{+2}$ ,  $\text{V}^{+2}$ ,  $\text{Mn}^{+3}$ ,  $\text{Mn}^{+2}$ ,  $\text{Fe}^{+3}$ ,  $\text{Fe}^{+2}$ ,  $\text{Co}^{+2}$ ,  $\text{Gd}^{+3}$  and  $\text{Dy}^{+3}$ . As noted, it is a 20 particularly advantageous feature that polymers are provided exhibiting a high substitution ratio, i.e., containing relatively large numbers of paramagnetic metal ions per molecule.

In the structure above,  $\text{m}$  is 0 or 1,  $\text{r}$  is 0 or 1,  $\text{a}$  is an integer preferably from 1 to 4,  $\text{b}$  is an integer 25 preferably from 0 to 3, and  $\text{d}$  is an integer preferably from 0 to 4. When  $\text{E}$  is present, i.e., when  $\text{m}$  is 1,  $\text{b}$  most preferably is 1.  $\text{d}$  can range up to about 100 when  $\text{Z}$  is the residue of multiple chelating groups. The total positive charge on the cations equals the sum of the total charge on 30 the residue of the chelating group plus the total charge on any counterions  $\text{E}$  present, i.e.,  $\text{a}=\text{d}+\text{b}$ .

The metal content in the polymer can vary from about 0.1 up to about 30% based on the total weight of the polymer. For MR imaging, the polymer preferably contains the paramagnetic metal in an amount of from 1 to 25%, more preferably 2-20% by weight.

For MR imaging, the polymer preferably comprises from 2 to about 100, more preferably 2 to 10 recurring units. If the polymer contains a peptide that can be hydrolyzed, then the polymer can contain from 1 to several hundred paramagnetic metal ions per molecule.

The polymer in structure I can be capped at the termini with groups independently selected from Z, Q, L or L<sub>1</sub> to which is bound a hydrogen atom or a linking group substituent such as described above. In preferred embodiments, wherein the polymer is a polyamide, the polymer can be capped with groups such as hydrogen or hydroxyl groups or with groups derived from polyamide chain terminating agents such as monoamines and monoacyl derivatives such as monoanhydrides, e.g., acetic anhydride, or with groups derived from the residue of a chelating group as defined above. It is further contemplated that cyclic polymers, i.e., non-capped polymers can be prepared.

The polymer of this invention can be prepared in water-soluble, water-dispersible or water-insoluble forms depending upon the intended application. The polymer can be crosslinked or non-crosslinked. The molecular weight of the polymer can vary widely, i.e., from about 1,000 to 10<sup>8</sup> or greater, as measured by gel permeation chromatography (GPC). The water-soluble polymer preferably is of molecular weight from 1,000 to about 250,000. The water-insoluble crosslinked polymer can be of molecular weight from about 10<sup>5</sup> to 10<sup>7</sup> or greater than . For MR imaging, the polymer preferably is of molecular weight 5,000-10<sup>6</sup>, more preferably 10,000-100,000.

The polymer of this invention can be prepared by contacting a reactive poly(alkylene oxide) species with a chelating agent or precursor thereof containing reactive functionality in a non-reactive solvent to form the polymer.

5 The poly(alkylene oxide) can be substituted or unsubstituted.

The preferred reaction conditions, e.g., temperature, pressure, solvent, etc., depend primarily on the particular reactants selected and can be readily determined by one skilled in the art.

10 Suitable reactive poly(alkylene oxide) species include terminally functionalized poly(alkylene oxide) diamines, poly(alkylene oxide) dihydrazines, poly(alkylene oxide) diisocyanates, poly(alkylene oxide) diols, poly(alkylene oxide) dialdehydes, poly(alkylene oxide) dicarboxylic acids, poly(alkylene oxide) bis(vinyl sulfone) ethers, poly(alkylene oxide) diphosphates, poly(alkylene oxide) N,N-dialkylaminophosphoramides, poly(alkylene oxide) diepoxides, poly(alkylene oxide) dialkoxides, poly(alkylene oxide) disulfonates, and the like. The above-described 15 poly(alkylene oxide) species are linear difunctional species. Tri- and higher multifunctional branched species relating to the above are also useful.

20 Suitable chelating agents and precursors thereof containing reactive functionality include polycarboxylic acids in dianhydride form, di(sulfonyl chlorides), di(alkyl sulfates), di(vinyl sulfones), and the like. As will be recognized by one skilled in the art, a suitably blocked pregenitor to the chelating agent or precursor thereof containing reactive functionality can be contacted with the 25 reactive poly(alkylene oxide) moiety to form the polymer, and then the blocking group can be subsequently removed by techniques known in the art. It is contemplated that 30 additional chelating functional groups can be introduced by

suitable chemical modification at the unblocked sites. If hydroxy substituents are to be selectively present in the final polymer, they must be temporarily blocked during polymerization, e.g., by conventional blocking techniques to 5 minimize formation of undesirable byproducts, e. g., polyester-amide derived therefrom. However, for some purposes, polyester-polyamides which contain one or more ester linking groups in the backbone of the polymer are contemplated to be useful.

10 In a preferred embodiment, the polymer of this invention can be prepared by reacting a linear poly(alkylene oxide) diamine with a precursor of a chelating agent in an internal dianhydride form.

15 The poly(alkylene oxide) diamine can be prepared by reacting an activated form of the poly(alkylene oxide) with ammonia, a primary amine, a polyamine or amide followed by reduction. The amino group can be introduced by other methods known in the art. Suitable illustrative amines include N-methylamine, amino acids, aminomethyl pyridine, 20 aminomethylthiophene, methoxyethoxyethylamine, methoxyethylamine and aminobenzoic acid. Exemplary useful polyamines include diaminohexane, tris(aminoethyl)amine, and diethylenetriamine.

25 The linear poly(alkylene oxide) in its diol form is widely available commercially or can be prepared by techniques well known to those skilled in the art. The poly(alkylene oxide) is activated for nucleophilic displacement by reacting it with an activator such as p-toluenesulfonyl chloride, thionyl chloride, thionyl bromide, 30 an alkylsulfonyl chloride, e.g.,  $\text{CH}_3\text{SO}_2\text{Cl}$ , a sulfonic acid anhydride, or any other suitable activator known in the art. The activated form of the poly(alkylene oxide) thus can be a ditosylate, a dichloride, a dibromide, etc.

The activated form of the poly(alkylene oxide) is reacted preferably with a stoichiometric excess of the amine, in an inert solvent preferably at a temperature, e.g., 100-160°C, and pressure, e.g., 1 to 10 atmospheres, sufficient to 5 drive the reaction to completion. Suitable solvents include dioxane, ethanol, and other alcohols. Thereafter, the poly(alkylene oxide) diamine preferably is isolated, e.g., by evaporation or precipitation, and purified, e.g., by dissolving in a suitable solvent such as methylene chloride, 10 chloroform or trichloroethane, and then washing the solution with an excess of aqueous NaOH, or by any other suitable isolation and purification techniques.

The internal anhydride forms of the chelating agents described above are commercially available and/or can 15 be prepared by techniques known in the art. For example, the internal anhydride forms of EDTA and DTPA are commercially available. The internal anhydride forms of DOTA, DO3A, OTTA, B4A, P4A and TMT can be prepared by techniques known in the art. For example, the anhydrides can be prepared by heating 20 the corresponding acids in acetic anhydride in the presence of pyridine as catalyst. Methods for the preparation of B4A, P4A and TMT are described in U.S. Patent 4,859,777. Mixed anhydrides are also suitable.

The reactive poly(alkylene oxide) diamine can be 25 reacted with the internal dianhydride in a non-reactive solvent to form the unmetallized polymer. The reaction conveniently can take place at approximately room temperature and atmospheric pressure. However, higher and lower temperatures and pressures are contemplated. Suitable 30 solvents include dimethylsulfoxide, dimethylformamide, acetonitrile, chloroform, dichloromethane and 1,2-dichloroethane. The nonmetallized polymer preferably is isolated and then purified, e.g., by diafiltration.

The metallized polymer can be formed by contacting the unmetallized polymer with a source of metal ions. This can be conveniently accomplished by adding a solution of or a solid salt or oxide of the metal to a solution, preferably an aqueous solution, of the polymer. Thereafter, the chelated polymer preferably is diafiltered in water to remove excess unchelated metal.

A general reaction scheme for this method of preparing the polymers of this invention and illustrative examples are set forth below.

Alternatively, the polymer can be prepared in a condensation polymerization reaction between a suitable diamine and a diacid containing the metallized chelating group, in a suitably activated form, e.g., in the form of an activated diester.

The molecular weight of the polymer product depends upon many factors including, for example, the molecular weight of the starting poly(alkylene oxide) moiety, the presence or absence of reactive polymerization chain terminating agents such as monoanhydrides or monoamines in the case of polyamides which reduce molecular weight by end-capping the polymer during the polymerization process, the presence or absence of reactive crosslinkers which increase the molecular weight of the polymer during polymerization, and the relative concentrations of the poly(alkylene oxide) and chelator present during the polymerization reaction which in turn affects the number of recurring units in the polymer product. To form the polymer of this invention in a water-insoluble form, the above described procedure can be modified to incorporate a crosslinkable tri- or higher polyamine, and/or by adding a reactive crosslinking agent, which can be the reactive chelating moiety, or, e.g., a diacid or higher acid chloride, to the polymerization reaction. The

preparation of insoluble and water-soluble polymers of molecular weight 1,000 to  $10^8$  can be accomplished by routine experimentation by one skilled in the art of polymer synthesis techniques.

5        In another embodiment, the polymer of this invention can comprise fluorocarbons, e.g., perfluoroalkyls, or trimethylsilyalkyl groups as substituents on the linking groups. For example, a fluoroalkyl amine can be incorporated as a substituent on the polyalkylene oxidyl group. In yet another embodiment, alkylene perfluorides can be incorporated onto the backbone of the polymer either in partial or full replacement of the units of the PEO. These groups reduce the viscosity of the polymers, which is advantageous primarily from the standpoint of formulating doses of the polymers for imaging. For example, PEG ditosylate can be treated with a perfluoro alkylene diol to form  $\text{-(PEO-O-}\text{CF}_2\text{)}_p\text{O-}y$  units, wherein  $p \geq 2$  to about 20 or more, and  $y$  is 1 to 100 or more, and PEO is as defined above. Alternatively, PEO diamines can be prepared from PEG ditosylate via reaction with fluorinated alkylamines, e.g.,  $\text{CF}_3\text{-}\{(\text{CH}_2\text{-})_w\text{NH}_2}$  wherein  $w$  is 0 or an integer from 1 to about 20. The resulting bis(fluoroalkylamino)-PEG can thereafter be converted into the desired chelating polymer using the techniques described above. In another embodiment, the PEG diamines can be alkylated with perfluoroalkylating agents, e.g.,  $\text{F-}\{(\text{CF}_2\text{-})_q\text{Br}$  wherein  $q$  is an integer from 1 to about 20. The use of perfluoro containing chelating agents is also contemplated, as is post polymerization perfluorination of other PEO-chelate copolymer systems.

30        The polymer preferably is prepared in a water soluble, i.e., injectable form when used as an MR contrast agent for blood pool imaging or as a composition intended to be administered intravenously. On the other hand, the

polymer can be prepared preferably as a water-insoluble polymer when intended to be administered orally as an MR contrast agent for gastro-intestinal imaging.

The dosages of the contrast agent used according to 5 the method of the present invention will vary according to the precise nature of the contrast agent used. Preferably however, the dosage should be kept as low as is consistent with achieving contrast enhanced imaging and volumes minimized for IV drip or bolus injection. In this way, the 10 toxicity potential is minimized. For most MR contrast agents the appropriate dosage will generally range from 0.02 to 3 mmol paramagnetic metal/kg body weight, especially 0.05 to 1.5 mmol/kg, particularly 0.08 to 0.5, more especially 0.1 to 0.4 mmol/kg. It is well within the skill of the average 15 practitioner in this field to determine the optimum dosage for any particular MR contrast agent by relatively routine experimentation, for both *in vivo* or *in vitro* applications.

Contrast agents may be formulated with conventional pharmaceutical or veterinary aids, for example stabilizers, 20 antioxidants, osmolality adjusting agents, buffers, pH adjusting agents, etc., and may be in a form suitable for injection or infusion directly or after dispersion in or dilution with a physiologically acceptable carrier medium, e.g., water for injection. Thus the contrast agents may be 25 formulated in conventional administration forms such as powders, solutions, suspensions, dispersions, etc., however solutions, suspensions and dispersions in physiologically acceptable carrier media will generally be preferred.

The contrast agents may be formulated for 30 administration using physiologically acceptable carriers or excipients in a manner fully within the skill of the art. For example, the compounds, optionally with the addition of pharmaceutically acceptable excipients, may be suspended or

dissolved in an aqueous medium, with the resulting solution or suspension then being sterilized.

PARENTERALLY ADMINISTRABLE FORMS, e.g., INTRAVENOUS SOLUTIONS, SHOULD OF COURSE BE STERILE AND FREE FROM

5 PHYSIOLOGICALLY UNACCEPTABLE AGENTS, AND SHOULD HAVE LOW OSMOLALITY TO MINIMIZE IRRITATION OR OTHER ADVERSE EFFECTS UPON ADMINISTRATION AND THUS THE CONTRAST MEDIUM SHOULD PREFERABLY BE ISOTONIC OR SLIGHTLY HYPERTONIC. SUITABLE VEHICLES INCLUDE AQUEOUS VEHICLES CUSTOMARILY USED FOR

10 ADMINISTERING PARENTERAL SOLUTIONS SUCH AS SODIUM CHLORIDE INJECTION, RINGER'S INJECTION, DEXTROSE INJECTION, DEXTROSE AND SODIUM CHLORIDE INJECTION, LACTATED RINGER'S INJECTION AND OTHER SOLUTIONS SUCH AS ARE DESCRIBED IN REMINGTON'S PHARMACEUTICAL SCIENCES, 15TH ED., EASTON: MACK PUBLISHING

15 CO., PP. 1405-1412 AND 1461-1487 (1975) AND THE NATIONAL FORMULARY XIV, 14TH ED. WASHINGTON: AMERICAN PHARMACEUTICAL ASSOCIATION (1975). THE SOLUTIONS CAN CONTAIN PRESERVATIVES, ANTIMICROBIAL AGENTS, BUFFERS AND ANTIOXIDANTS CONVENTIONALLY USED FOR PARENTERAL SOLUTIONS, EXCIPIENTS AND OTHER ADDITIVES

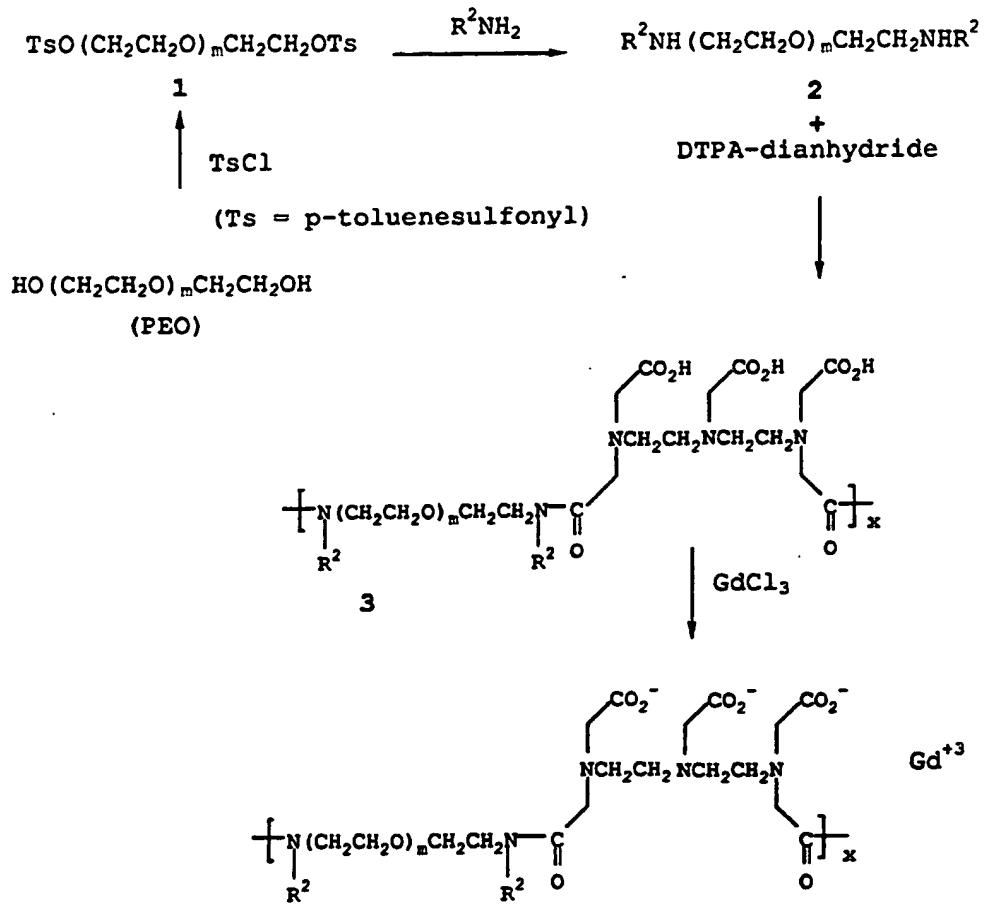
20 WHICH ARE COMPATIBLE WITH THE CONTRAST AGENTS AND WHICH WILL NOT INTERFERE WITH THE MANUFACTURE, STORAGE OR USE OF PRODUCTS.

THE FOLLOWING EXAMPLES FURTHER ILLUSTRATE THE INVENTION.

25 EXAMPLES 1-12 ILLUSTRATE THE PREPARATION OF NON-CROSSLINKED POLYMERS OF THE INVENTION.

Example 1

30 A POLYMER OF THE INVENTION (Ia) WAS PREPARED IN ACCORDANCE WITH REACTION SCHEME A AS DESCRIBED BELOW.



I a

A solution of 100.0 g (0.0690 mol) of PEO of average molecular weight (MW) 1450 in toluene (1500 ml) was refluxed for 2 hours with azeotropic removal of water. The 5 solution was cooled to 25°C, then treated with triethylamine (46.1 ml, 0.331 mol), 4-dimethylaminopyridine (1.69 g, 0.0138 mol) and *p*-toluenesulfonyl chloride (57.9 g, 0.303 mol), and then heated for 4 days at 60°C under an atmosphere of nitrogen. After cooling to room temperature, the reaction 10 mixture was filtered and the filtrate was extracted twice

with water. The combined aqueous extracts were washed with ether, then extracted twice with CHCl<sub>3</sub>. The combined CHCl<sub>3</sub> extracts were dried over anhydrous magnesium sulfate and then concentrated to yield 121.3 g of product (1).

5        A solution of 42.2 g (0.0240 mol) of the ditosylate 1 in 420 ml of dioxane was cooled in an ice bath and a stream of methylamine was introduced over a period of 35 minutes. The reaction mixture was then heated in a stainless steel reactor at 160°C for 16 hours, cooled to room temperature, 10 and then filtered. The filtrate was concentrated to remove solvent, then treated with water (844 ml) and 1.0 N NaOH (95.2 ml) and extracted twice with CHCl<sub>3</sub>. The combined CHCl<sub>3</sub> extracts were dried over anhydrous magnesium sulfate and concentrated to leave 31.0 g. of product (2) (R<sup>2</sup>=CH<sub>3</sub>).

15        A solution of 9.00 g (6.10 mmol) of the bis-(N-methylamine) 2 in 45 mls of dimethylsulfoxide (DMSO) was treated with triethylamine (1.70 ml, 12.2 mmol) and a solution of 2.18 g (6.10 mmol) of diethylenetriaminepentaacetic acid internal dianhydride in 20 DMSO (45 mls). The reaction mixture was stirred at room temperature for 16 hours, then treated with 360 ml of water. The resultant solution was filtered through a 0.45 µm nylon filter and the filtrate was diafiltered against water in a diafiltration cell equipped with a 3000 MW cut-off membrane 25 to leave 170 ml of a solution of (3) (R<sup>2</sup>=CH<sub>3</sub>).

30        A 160 ml portion of the aqueous solution was treated with a two-fold molar excess of gadolinium(III) chloride hexahydrate, and then was diafiltered against water as described above. Lyophilization of the retentate yielded 8.66 g of product (Ia) (R<sup>2</sup>=CH<sub>3</sub>) of average MW 16,300 daltons (as determined by SEC-HPLC using PEO molecular weight standards). Elemental analysis for C<sub>82</sub>H<sub>156</sub>GdN<sub>5</sub>O<sub>40</sub>·4H<sub>2</sub>O:

<u>Element</u>	<u>% Theory</u>	<u>% Found</u>
C	47.32	47.15
H	7.94	7.89
N	3.36	3.30
5 Gd	7.55	7.37

The relaxivity ( $T_1$ ) $^{-1}$  of this material at 20 MHz and 40°C was found to be 6.2 mM $^{-1}$ s $^{-1}$ .

10 Intravenous administration of 100, 200 and 400 mg/Kg to mice resulted in no deaths, no effect on body weight and no abnormalities upon necropsy after 14 days.

The same product, but prepared using radioactive  $^{153}\text{Gd}$  was employed in biodistribution studies in rats to determine a blood-pool half-life (elimination phase) of 75 15 minutes.

#### Example 2

20 In a manner similar to Example 1, a polymeric gadolinium chelate (Ia,  $\text{R}^2 = \text{CH}_3$ ) of average MW 8,010 was prepared from PEO of MW 1000. The blood-pool half-life (elimination phase) was determined to be 48 minutes.

#### Example 3

25 In a manner similar to Example 1, a polymeric gadolinium chelate (Ia,  $\text{R}^2=\text{CH}_3$ ) of average MW 16,800 was prepared from PEO of average MW 2000.

#### Example 4

In a manner similar to Example 1, a polymeric gadolinium chelate (Ia,  $\text{R}^2 = \text{CH}_3$ ) of average MW 22,400 was

prepared from PEO of average MW 3350. Elemental analysis for  $C_{168}H_{328}GdN_5O_{83}\cdot 5H_2O$ :

	<u>Elemental</u>	<u>% Theory</u>	<u>% Found</u>
5	C	50.00	50.00
	H	8.53	8.61
	N	1.75	1.71
	Gd	3.94	3.78

10 The blood-pool half-life (elimination phase) of this material in rats was determined to be 141 minutes.

Example 5

15 This example describes the preparation of a polymer (Ia) wherein  $R^2=H$ .

10 A solution of 15.30 g (11.70 mmol) of ditosylate 1 prepared from PEO of average MW 1000 in 153 ml of absolute ethanol was cooled in an ice bath, and a stream of ammonia was introduced over a period of 30 minutes. The reaction mixture was heated in a stainless steel reactor at 100°C for 16 hr, cooled to room temperature, and then filtered. The filtrate was concentrated to remove solvent, treated with water (153 ml) and 1.0 N NaOH (46.8 ml), and extracted twice with  $CHCl_3$ . The  $CHCl_3$  extracts were dried over anhydrous magnesium sulfate, filtered and then concentrated to leave 12.20 g of product (2) ( $R^2=H$ ).

30 A solution of 11.22 g (11.24 mmol) of the diamine (2) in 56 ml of DMSO was treated with triethylamine (3.13 ml, 22.5 mmol) and a solution of 4.017 g (11.24 mmol) of diethylenetriaminepentaacetic acid dianhydride in DMSO (56 ml). The reaction mixture was stirred at room temperature for 16 hr, and then treated with 448 ml of water. The

resulting solution was filtered through a 0.45  $\mu\text{m}$  filter and the filtrate was diafiltered against water in a diafiltration cell equipped with a 3000 MW cut-off membrane to leave 225 ml of solution.

5 A 208 ml portion of the aqueous solution was treated with a two-fold excess of gadolinium (III) chloride hexahydrate, and then diafiltered against water.

Lyophilization of the retentate yielded 11.58 g of product (Ia,  $\text{R}^2=\text{H}$ ) of average MW 12,500. Elemental analysis for  
10  $\text{C}_{60}\text{H}_{112}\text{GdN}_5\text{O}_{30}\cdot 2\text{H}_2\text{O}$ :

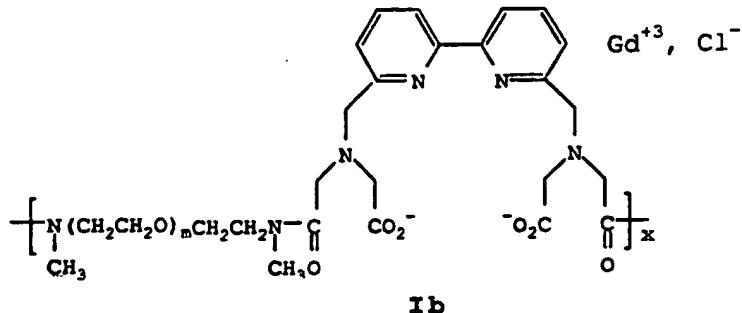
	<u>Element</u>	<u>%Theory</u>	<u>%Found</u>
	C	45.70	45.95
	H	7.42	7.53
15	N	4.44	3.85
	Gd	9.97	10.20

Example 6

20 Example 5 was repeated except that the starting PEO had an average MW of 1450. The lyophilized product was determined to have an average MW of 21,900.

Example 7

25 Example 1 was repeated except that B4A-dianhydride was used in place of DTPA-dianhydride. The product (Ib) was determined to have an average MW of 17,600.



Elemental analysis for  $C_{88}H_{156}ClGdN_6O_{38} \cdot 4H_2O$ :

5

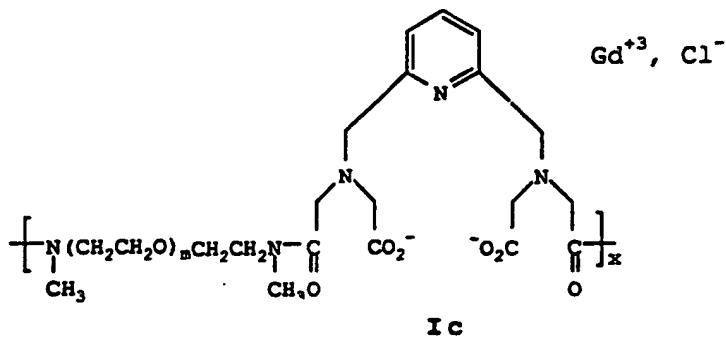
	<u>Element</u>	<u>%Theory</u>	<u>%Found</u>
	C	48.69	48.56
	H	7.61	7.58
	N	3.87	3.76
10	Gd	7.24	7.09

Example 8

15

Example 1 was repeated except that P4A-dianhydride was used in place of DTPA-dianhydride. The product (Ic) was determined to have an average MW of 20,000.

20



Elemental analysis for  $C_{83}H_{153}ClGdN_5O_{38} \cdot 4H_2O$ :

	<u>Element</u>	<u>%Theory</u>	<u>%Found</u>
5	C	47.61	47.38
	H	7.75	7.96
	N	3.34	3.21
	Gd	7.51	7.46

Example 9

10 Example 1 was repeated except that  $DyCl_3$  was used in place of  $GdCl_3$ . The lyophilized product was found to have an average MW of 14,800. The relaxivity ( $T_2$ ) $^{-1}$  of this material at 20 MHZ and 40°C was found to be  $0.109\text{ mM}^{-1}\text{s}^{-1}$ . Elemental analysis for  $C_{82}H_{156}DyN_5O_{40}$ :

	<u>Element</u>	<u>%Theory</u>	<u>%Found</u>
15	C	48.89	48.76
	H	7.80	7.87
	N	3.48	3.48
	Dy	8.07	7.80

Example 10

25 Example 9 was repeated except that the starting PEO had an average MW of 2000. The lyophilized product was found to have an average molecular weight of 15,300. Elemental analysis for  $C_{106}H_{204}DyN_5O_{52}\cdot 4H_2O$ :

	<u>Element</u>	<u>%Theory</u>	<u>%Found</u>
30	C	48.46	48.71
	H	8.17	8.05
	N	2.68	2.52
	Dy	6.21	6.04

Example 11

Example 9 was repeated except that the starting PEO had an average MW of 3350. The lyophilized product was found 5 to have an average molecular weight of 20,100. Elemental analysis for  $C_{168}H_{328}DyN_5O_{83}\cdot H_2O$ :

	<u>Element</u>	<u>%Theory</u>	<u>%Found</u>
10	C	51.15	50.93
	H	8.48	8.45
	N	1.78	1.80
	Dy	4.12	4.05

Example 12

15 Example 5 was repeated except that  $DyCl_3$  was used in place of  $GdCl_3$ . The lyophilized product was found to have an average MW of 45,500. The relaxivity  $(T_2)^{-1}$  of this material at 20 MHZ and 40°C was found to be 0.122 20  $mm^{-1}s^{-1}$ . Elemental analysis for  $C_{80}H_{152}DyN_5O_{40}\cdot 4H_2O$ :

	<u>Element</u>	<u>%Theory</u>	<u>%Found</u>
25	C	46.68	46.75
	H	7.83	7.69
	N	3.40	3.15
	Dy	7.89	8.04

Examples 13-16 illustrate the preparation of crosslinked polymers of the invention.

30

Example 13

A solution of 15.45 g (8.788 mmol) of ditosylate (1) (prepared from PEO of average MW 1450) in 155 mls of dioxane was treated with 20.4 g (0.176 mol) of 1,6-hexanediamine. The reaction mixture was heated in a stainless steel reactor at 160°C for 16 hours. The cooled reaction mixture was concentrated to remove solvent, and then treated with water (309 ml) and 1.0 N NaOH (35.2 ml). The aqueous solution was washed twice with ether, and then extracted twice with CHCl<sub>3</sub>. The combined CHCl<sub>3</sub> extracts were dried over anhydrous magnesium sulfate, filtered and concentrated at 80°C at 0.5 mm Hg to remove solvent and excess 1,6-hexanediamine and to leave 12.63 g of product (2) (R<sup>2</sup>=H<sub>2</sub>N(CH<sub>2</sub>)<sub>6</sub>).

A solution of 4.00 g (2.43 mmol) of (2) (R<sup>2</sup>=H<sub>2</sub>N(CH<sub>2</sub>)<sub>6</sub>) in 44 ml of DMSO was treated with triethylamine (1.35 ml, 9.72 mmol) and a solution of 0.867 g (2.43 mmol) of diethylenetriaminepentaacetic acid dianhydride in DMSO (48 ml). The reaction mixture was stirred at room temperature for 16 hours, and then treated with 384 ml of water. The resulting solution was filtered through a 0.45 µm nylon filter and the filtrate was diafiltered against water in a diafiltration cell equipped with a 10,000 MW cut-off membrane.

The retentate aqueous polymer solution was treated with a two-fold excess of gadolinium(III) chloride hexahydrate and then diafiltrated against water as described above. Lyophilization yielded 2.10 g of cross-linked product of average MW 49,800 and containing 8.03% Gd by weight.

Example 14

In a manner similar to Example 13, a cross-linked polymeric gadolinium chelate of average MW 36,200 containing 5 9.30% Gd by weight was prepared from a solution of 3.31 g (2.01 mmol) of (2) ( $R^2=-(CH_2)_6NH_2$ ) in 76 ml of DMSO, triethylamine (1.12 ml, 8.04 mmol) and a solution of 1.078 g (3.016 mmol) of diethylenetriaminepentaacetic acid dianhydride in DMSO (79 ml).

10

Example 15

In a manner similar to Example 13, a cross-linked polymeric gadolinium chelate of average MW 95,300 containing 15 11.30% Gd by weight was prepared from a solution of 3.00 g (1.82 mmol) of (2) ( $R^2=-(CH_2)_6NH_2$ ) in 69 ml of DMSO, triethylamine (1.02 ml, 7.29 mmol) and a solution of 1.303 g (3.645 mmol) of diethylenetriaminepentaacetic acid dianhydride in DMSO (72 ml). The relaxivity ( $T_1$ )<sup>-1</sup> of this 20 material at 20 MHz and 40°C was found to be 8.55 mM<sup>-1</sup>s<sup>-1</sup>.

Example 16

In a manner similar to Example 13, ditosylate (1) 25 (prepared from PEO of average MW 1450) was reacted with tris(2-aminoethyl)amine to yield (2) ( $R^2=CH_2CH_2N(CH_2CH_2NH_2)_2$ ).

A cross-linked polymeric gadolinium chelate of average MW 41,400 was prepared from a solution of 0.80 g (0.47 mmol) of (2) ( $R^2=CH_2CH_2N(CH_2CH_2NH_2)_2$ ) in 8 ml of DMSO, 30 triethylamine (0.39 ml, 2.8 mmol) and a solution of 0.34 g (0.94 mmol) of diethylenetriaminepentaacetic acid dianhydride in DMSO (8 ml). The relaxivity ( $T_1$ )<sup>-1</sup> of this material at 20 MHz and 40°C was found to be 10.2 mM<sup>-1</sup>s<sup>-1</sup>.

## MR IMAGING STUDIES

The compositions of Examples 1, 2, 4, 5 and 6 were imaged in a Rabbit V-2 (carcinoma) Tumor Model as follows. The concentration of each composition was adjusted to 0.1 mmol/kg Gd, i.e., the concentrations were adjusted respectively to 102 mM, 124 mM, 49 mM, 131 mM, and 53.5 mM solutions. For each example, 3 rabbits were employed. Rabbits were anesthetized, injected and imaged on a standard magnetic resonance imaging device. An axial pre-contrast and post-contrast scans at time intervals of t=0 (immediately after injection), 15 min, 30 min, 60 min and 24 hr were made in 3 mm slices (5 mm apart) of areas from the liver to the legs. Plots of relative enhancement vs. time were derived from three regions of interest (ROI) from enhancing tumor in the right leg, three ROI from enhancing tumor in the left leg and one ROI from muscle.

As is indicated by the plots in Figure 1 and the images of Figures 2 and 3, the compositions of this invention exhibited outstanding image enhancement and dramatically improved uptake in the tumor model as compared to the Magnevist control.

Example 17 - Synthesis of the Gd(III) Complex of the Polyamide of 1,11-diamino-3,6,9-trioxaundecane and Diethylenetriaminepentaacetic acid.

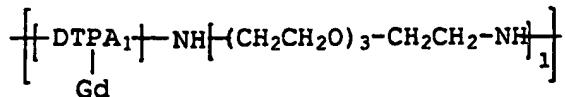
a. Polymerization  
To a solution of 5.72 g (30.0 mmol) of 1,11-diamino-3,6,9-trioxaundecane and 7.56 g (90 mmol) of sodium hydrogen carbonate in 212 ml of dimethyl sulfoxide and 13 ml of water was added 11.37 g (31.8 mmol) of diethylenetriaminepentaacetic dianhydride with vigorous

stirring. The resulting reaction mixture was stirred at room temperature for 5 hr following which it was diluted to approximately 1% solids with water and diafiltrated for 6 turnovers using a nominal 10,000 molecular weight cutoff, 5 spiral wound, polysulfone diafiltration membrane. The resulting aqueous retentate was then freeze-dried, yielding a fluffy white solid (7.86 g).

b. Complex Formulation

A total of 7.0 g of the above polymeric solid was 10 dissolved in 140 ml of deionized water and stirred at a moderate speed, as it was slowly treated with a 5% aqueous solution of gadolinium (III) chloride hexahydrate. The addition was continued until a small test sample, dripped into PAR test reagent, caused a color change from pale yellow 15 to deep yellow. The PAR test reagent had been prepared previously by sonicating a mixture of 40 ml deionized water, 20 ml of trace metal grade ammonium hydroxide, and 0.005 g of 4-(2-pyridylazo) resorcinol for 1 min. Following sonication, it was treated with 5.7 ml of trace metal grade acetic acid, 20 allowed to cool to room temperature, and diluted to 100.0 ml with additional deionized water.

Upon observing the color change in the PAR reagent, the polymer complex was diafiltrated as before for another 4 turnovers, following which the pH was adjusted to 6.5 with a 25 1.0 molar sodium hydroxide. It was then freeze-dried producing a fluffy white solid. This ligand complex was found to have a weight average molecular weight of 22,000, a number average molecular weight of 15,200, and a polymer dispersity of 1.45. It was shown to contain 21.1 wt. % bound 30 gadolinium and 0.001 wt. % free gadolinium. The polymer has the recurring units of the structure



Example 18 Synthesis of the Gd(III) Complex of the Polyamide of 1,8-diamino-3,6-dioxaoctane and  
5 Diethylenetriaminepentaacetic acid.

a. Polymerization

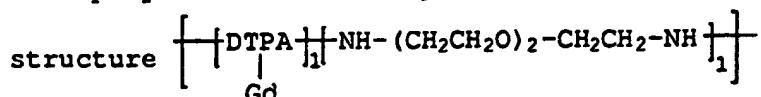
To a solution of 3.70 g (25.0 mmol) of 1,8-diamino-3,6-dioxaoctane and 6.3 g (75.0 mmol) of sodium hydrogen carbonate in 28 ml of dimethyl sulfoxide and 5 ml of water 10 was added 10.35 g (29.0 mmol) of diethylenetriaminepentaacetic dianhydride with vigorous stirring. The resulting reaction mixture was heated in oil bath at 90° C for 30 min, and the solution was continuously stirred at room temperature for 3 hr, following which it was 15 diluted to approximately 1% solids with water and diafiltrated for 6 turnovers using a nominal 10,000 molecular weight cutoff, spiral wound, polysulfone diafiltration membrane. The resulting aqueous retentate, comprising 800 ml, was then used directly to prepare the gadolinium complex as described 20 below.

b. Complex Formulation

The polymer solution (600 ml) was stirred at moderate speed, as it was slowly treated with a 5% aqueous solution of gadolinium (III) chloride hexahydrate. The addition was 25 continued until a small test sample, dripped into PAR test reagent caused a color change from pale yellow to deep yellow. The PAR test reagent was prepared as described in Example 17.

Upon observing the color change in the PAR reagent, the 30 polymer complex was diafiltrated as before for another 4 turnovers, following which the pH was adjusted to 7.5 with

1.0 molar sodium hydroxide. It was then freeze-dried, producing a fluffy white solid (4.40 g). This ligand complex was found to have a weight average molecular weight of 14,400, a number average molecular weight of 10,800, and a 5 polymer dispersity of 1.33. It was shown to contain 20.2 wt. % bound gadolinium and 0.004 wt. % free gadolinium. This polymer has recurring units of the



10 **Example 19** Synthesis of the Gd(III) Complex of the Polyamide of 4,7,10-trioxa-1,13-tridecanediamine and Diethylenetriaminepentaacetic acid.

a. Polymerization

15 To a solution of 5.50 g (25.0 mmol) of 4,7,10-trioxa-1,13-tridecanediamine and 6.3 g (75.0 mmol) of sodium hydrogen carbonate in 30 ml of dimethylsulfoxide and 5 ml of water was added 10.35 g (29.0 mmol) of diethylenetriaminepentaacetic dianhydride with vigorous 20 stirring. The resulting reaction mixture was heated in oil bath at 75° C for 30 min following which it was diluted to approximately 1% solids with water and diafiltrated for 6 turnovers using a nominal 10,000 molecular weight cutoff, spiral wound, polysulfone diafiltration membrane. The 25 resulting aqueous retentate, comprising 800 ml was then used directly to prepare the gadolinium complex as described below.

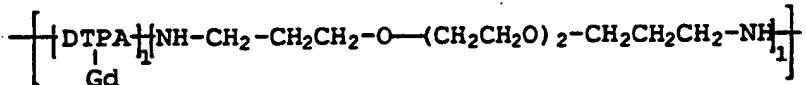
b. Complex Formation

30 The aqueous retentate from above (600 ml) was stirred at moderate speed, as it was slowly treated with a 5% aqueous solution of gadolinium (III) chloride hexahydrate. The

additon was continued until a small test sample, dripped into PAR test reagent, prepared as described above, caused a color change from pale yellow to deep yellow.

Upon observing the color change in the PAR reagent, the 5 polymer complex was diafiltered as before for another 4 turnovers, following which the pH was adjusted to 7.5 with 1.0 molar sodium hydroxide. It was then freeze-dried, producing a fluffy white solid (4.40 g). This ligand complex was found to have a weight average molecular weight of 10 17,900, a number average molecular weight of 13,600, and a polymer dispersity of 1.32. It was shown to contain 20.1 wt. % bound gadolinium and 0.003 wt. % free gadolinium.

This polymer has recurring units of the structure:



15 The polymers prepared in Examples 17-19 each provided excellent image enhancement in MR imaging studies.

Example 20 Alternative Synthesis of Polymer of the Invention

20

Step 1 Polyoxyethylene bis(chloride)

To a solution of polyethylene glycol (Av. Mol. Wt. 14,500) (1000 g; 0.69 mole) in 1.5 L toluene (60-80°C) was added  $\text{SOCl}_2$  (200 ml; 2.76 mol) and DMF (10ml) through a 25 dropping funnel over 10 min. The reaction mixture was heated on a steam bath for 1 hr. An analysis by TLC ( $\text{CH}_2\text{Cl}_2:\text{MeOH}$ ; 6:1) indicated a very small amount of starting material still remained. An additional 20 ml of  $\text{SOCl}_2$  was added to the reaction and it was heated on the steam bath for another 20 30 minutes to drive the reaction to completion. After cooling the reaction to 0° C with an ice bath, 1N NaOH (2.5L) was

carefully introduced to neutralize the solution and the layers were separated. The aqueous layer was washed with CH<sub>2</sub>Cl<sub>2</sub> (3 x 1L) and the combined CH<sub>2</sub>Cl<sub>2</sub> layer was washed with water (2 x 1L) and dried with MgSO<sub>4</sub>. It was filtered and 5 concentrated under reduced pressure to give a light yellow oil. The product was precipitated out by adding tert-butyl methyl ether (TBME) (2L) to the residue with cooling and stirring. The filtered product was dried in the oven under vacuum overnight to yield 941 g (94%) of the titled compound 10 as a white solid. An analysis by HPLC indicated the purity of the product was 99.86% with no oligomer. Anal. Cal'd/Found for C<sub>64</sub>H<sub>128</sub>O<sub>31</sub>Cl<sub>2</sub>: C, 52.49/51.94; H, 8.81/8.43; Cl, 4.84/5.00.

15 Step 2 Polyoxyethylene bis(azide)

To a suspension of polyoxyethylene bis(chloride) (500 g; 0.336 mol) and KI (139 g; 0.841 mol) in 1500 mL DMF was added NaN<sub>3</sub> (109 g; 1.681 mol). The suspension was heated on a steam bath at 70° C for 12 hours to give a yellowish 20 solution. After cooling the reaction to room temperature, 2.5 L of water was added and the solution was extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 x 1L). The combined CH<sub>2</sub>Cl<sub>2</sub> layer was washed with water (3 x 1L), then dried (MgSO<sub>4</sub>), filtered and concentrated under reduced pressure to give a light yellow oil. The 25 product was precipitated out by adding TBME (1L) to the residue with cooling and stirring. The filtered product was dried in the oven under vacuum overnight to yield 452 g (90%) of the PEG diazide as a white solid. An analysis by HPLC indicated the purity of the product was 99.2% with no 30 oligomer.

Anal. Calc'd/Found for C<sub>64</sub>H<sub>128</sub>O<sub>31</sub>N<sub>6</sub>: C, 52.09/52.50; H, 8.73/8.58; N, 5.69/5.11.

**Step 3 Polyoxyethylene bis(amine)**

To a solution of polyoxyethylene bis(azide) (176 g; 0.117 mol) in 1 L 1N HCl was added Pd/C (17.6 g). The suspension was hydrogenated at 45 PSI for 15 hours. After an 5 analysis by TLC ( $\text{CH}_2\text{Cl}_2:\text{MeOH}$ ; 4:1) on an aliquat withdrawn from the reaction indicated no more starting material, the catalyst was removed by carefully filtering through a short pad of Celite. The filtrate was neutralized with 35% NaOH to pH>10, then extracted with  $\text{CH}_2\text{Cl}_2$  (2 x 600 ml). The combined 10  $\text{CH}_2\text{Cl}_2$  layer was washed with water (1 L) and concentrated under reduced pressure to give a light yellow oil (165 g). The residue was redissolved in 1 L of water and 150 ml of HOAc was added. The acidic solution was extracted with  $\text{CH}_2\text{Cl}_2$  (3 x 500 ml) to remove most impurities and neutralized with 15 35% NaOH to pH>10. The neutralized aqueous solution was extracted with  $\text{CH}_2\text{Cl}_2$  (2 x 500 ml) and the  $\text{CH}_2\text{Cl}_2$  layer was washed with water (500 ml). Then it was dried ( $\text{MgSO}_4$ ), filtered and concentrated under reduced pressure to give a light yellow oil. The product was precipitated out by adding 20 TBME (1 L) to the residue with cooling and stirring. The filtered produce was dried in the oven under vacuum overnight to yield 138 g (81%) of the titled compound as a white solid. An analysis by HPLC indicated the purity of the product was 96.9% with no oligomer (detected by gel chromatography). 25 Anal. Calc'd/Found for  $\text{C}_{64}\text{H}_{132}\text{O}_{31}\text{N}_2$ : C, 53.92/53.24; H 9.33/9.35; N 1.96/1.66.

**Step 4 PEG-DTPA polymer**

To a solution of polyoxyethylene bis(amine) (103 g; 30 0.071 mol) in 1545 ml  $\text{CH}_3\text{CN}$  was added triethylamine (23 ml; 0.142 mol) and DTPA bis(anhydride) (28 g; 0.078 mol). The suspension was stirred at room temperature for 1/2 hour and a clear solution developed. The reaction was stirred for an

additional 2 hours and 15 minutes and 3.5 L of ether was added. A precipitate was quickly observed. The mixture was stirred for 10 minutes and the solvent was decanted. After drying the precipitate, 113 g of product was obtained. An 5 analysis by HPLC indicated the product had Av. Mol. Wt. 16,800 and polydispersity 1.80, which was directly used for preparation of the chelated polymer in a manner similar to that described above.

In addition to their demonstrated specificity to 10 accumulation in tumors and sites of infection, the polymers of this invention are expected to be effective in imaging the spleen and blood brain barrier defects; and in imaging perfusion defects such as those found in ischemic tissues.

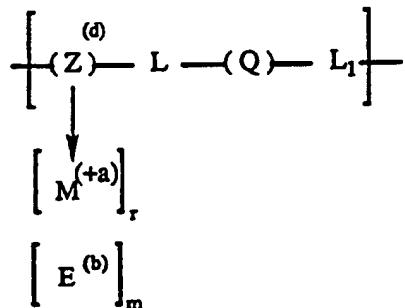
The invention has been described in detail with 15 particular reference to certain preferred embodiments thereof, but it will be understood that variations and modifications can be effected within the spirit and scope of the invention.

## Claims:

1. A polymer suitable for MR imaging containing units comprising the residue of a chelating agent linked to a 5 poly(alkylene oxide) moiety, said chelating agent having a paramagnetic metal ion associated therewith.

2. The polymer of claim 1 wherein said units have the structure

10



wherein:

15 Z is the residue of a chelating agent;  
 Q is a poly(alkylene oxide) moiety;  
 M^{+a} is one or more cations having a total charge of  
 +a;  
 L and L<sub>1</sub> independently represent a chemical bond or a  
 linking group;  
 E^{(b)} is one or more counterions having a total charge  
 20 of b;  
 m is 0 or 1;  
 r is 0 or 1;  
 d is the total charge on the linked residue of the  
 chelating group; and  
 25 a = d+b.

3. The polymer of claim 1 wherein said polymer comprises from 2 to 1000 of said units.

4. The polymer of claim 2 wherein 2 is the residue of 5 a chelating agent selected from the group consisting of EDTA, DTPA, DOTA, DO3A, OTTA, CDTPA, P4A, B4A, PheMT, DCDTPA and TMT.

5. The polymer of claim 2 wherein Q is selected from 10 the group consisting of a poly(ethylene oxide) moiety, a poly(propylene oxide) moiety and a poly(ethylene oxide)-co-poly(propylene oxide) moiety of MW 250-1000.

6. The polymer of claim 2 wherein M<sup>+a</sup> is selected from 15 the group consisting of Gd<sup>+3</sup>, Fe<sup>+3</sup>, Mn<sup>+2</sup>, Mn<sup>+3</sup>, Dy<sup>+3</sup> and Cr<sup>+3</sup>.

7. The polymer of claim 2 wherein L and L<sub>1</sub> 20 independently represent amino, imido, nitrilo, imino, alkylene, carbonyl, sulfonyl, sulfinyl, ether, thioether, ester, thioester, amide, thioamide, thio, dithio, phosphate, phosphonate, urelene, thiourelene, urethane, thiourethane, an amino acid linkage or a peptide linkage.

8. The polymer of claim 2 wherein L and L<sub>1</sub> 25 independently represent an imino or amide linkage.

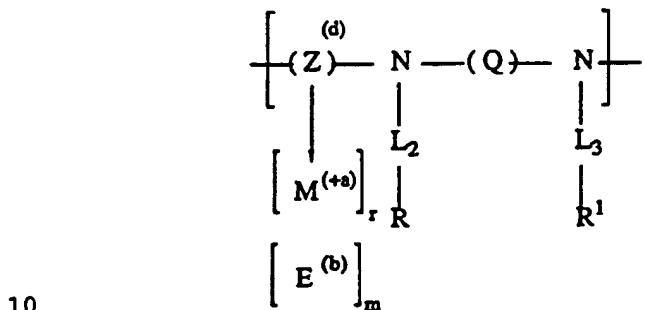
9. The polymer of claim 2 wherein L and L<sub>1</sub> independently represent

30 
$$\begin{array}{c} \text{---N---} \quad \text{and} \quad \text{---N---} \\ | \quad \quad \quad | \\ \text{L}_2 \quad \quad \quad \text{L}_3 \\ | \quad \quad \quad | \\ \text{R} \quad \quad \quad \text{R}' \end{array}$$
  
L<sub>2</sub> and L<sub>3</sub> independently represent a chemical bond

or a linking group;

R and R<sup>1</sup> independently are H, OH, alkyl, aryl, aralkyl, alkoxy, heterocyclyl, a carboxyl group, an ester group, the residue of a chelating group or a poly(alkylene oxide) moiety.

10. The polymer of claim 1 wherein said units are recurring units having the structure



wherein:

Z is the residue of a chelating agent;

Q is a poly(alkylene oxide) moiety,

15 M<sup>(+a)</sup> is one or more cations having a total charge of +a;

L<sub>2</sub> and L<sub>3</sub> independently represent a chemical bond or a linking group;

20 R and R<sup>1</sup> independently are H, OH, alkyl, aryl, halogenated aryl, aralkyl, halogenated aralkyl, alkoxy, heterocyclyl, a carboxyl group, a carboxylate group, an ester group, the residue of a chelating group or a poly(alkylene oxide) moiety;

25 E<sup>(b)</sup> is one or more counterions having a total charge of b;

m is 0 or 1;

r is 0 or 1;

d is the total charge on the residue of the chelating group, and  
a=d+b.

5

11. The polymer of claim 10 wherein Z is the residue of B4A,  $M^{+a}$  is  $Gd^{+3}$  or  $Dy^{+3}$ ,  $E^b$  is  $Cl^-$ ,  $L_2$  and  $L_3$  represent a chemical bond, Q is a poly(ethylene oxide) moiety of MW 250-10,000, and R and  $R^1$  are  $CH_3$ .

10

12. The polymer of claim 10 wherein Z is the residue of P4A,  $M^{+a}$  is  $Gd^{+3}$  or  $Dy^{+3}$ ,  $E^b$  is  $Cl^-$ ,  $L_2$  and  $L_3$  represent a chemical bond, Q is a poly(ethylene oxide) moiety of MW 250-10,000, and R and  $R^1$  are  $CH_3$ .

15

13. The polymer of claim 10 wherein Z is the residue of DTPA,  $M^{+a}$  is  $Gd^{+3}$  or  $Dy^{+3}$ ,  $L_2$  and  $L_3$  represent a chemical bond, Q is a poly(ethylene oxide) moiety of MW 250-10,000, and R and  $R^1$  are H or  $CH_3$ .

20

14. The polymer of claim 2 wherein Q is  $-(CH_2CH_2O)_mCH_2CH_2-$  and m is 1 to 5000.

25

15. The polymer of claim 2 wherein Q is  $-(CH_2CH_2O)_mCH_2CH_2-$  and m is 1 to 6.

16. The polymer of claim 15 wherein m is 2 or 3.

30

17. The polymer of claim 2 wherein Q is selected from the group consisting of  $-CH_2CH_2O\rightarrow_3CH_2CH_2-$ ;  $-CH_2CH_2O\rightarrow_2CH_2CH_2-$ ; or  $-CH_2\{CH_2CH_2O\rightarrow_3CH_2CH_2CH_2-$ .

18. A method of performing an MR diagnostic procedure

in a human or non-human body comprising administering to the body a contrast enhancing amount of the polymer of claim 1 and then exposing the body to an MR measurement step to image at least a portion of the body.

5

19. The method of claim 18 wherein the polymer is administered with a pharmaceutically acceptable carrier.

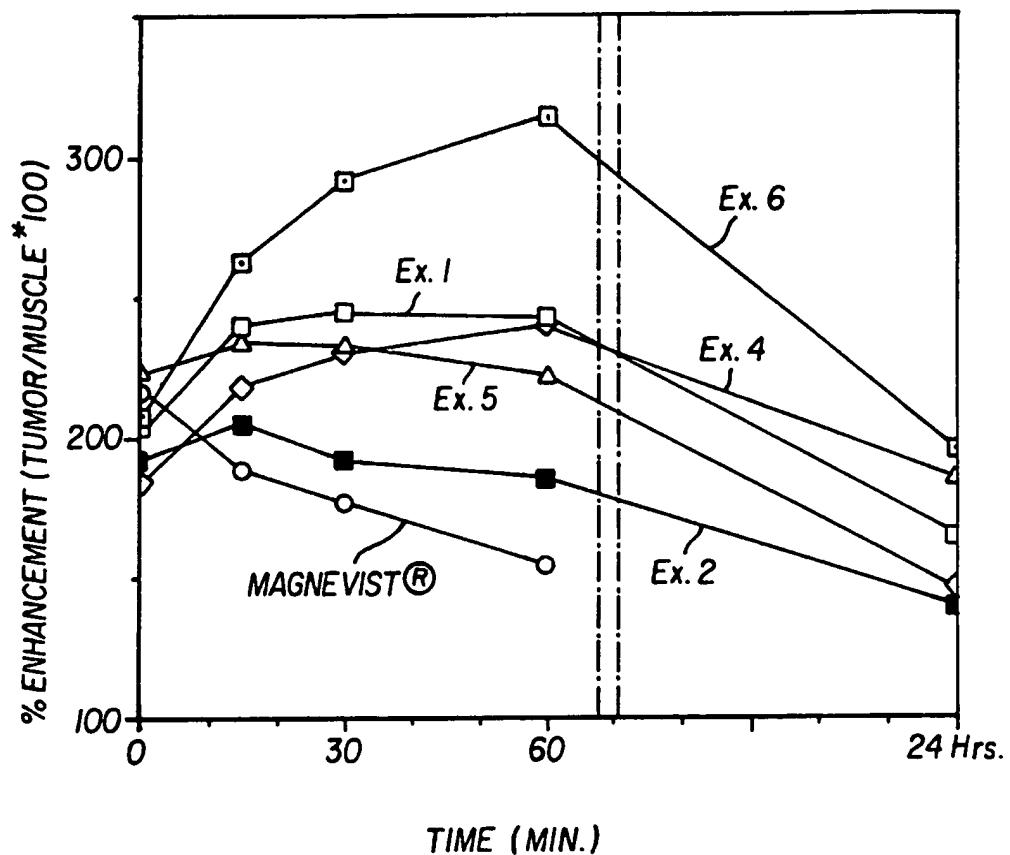
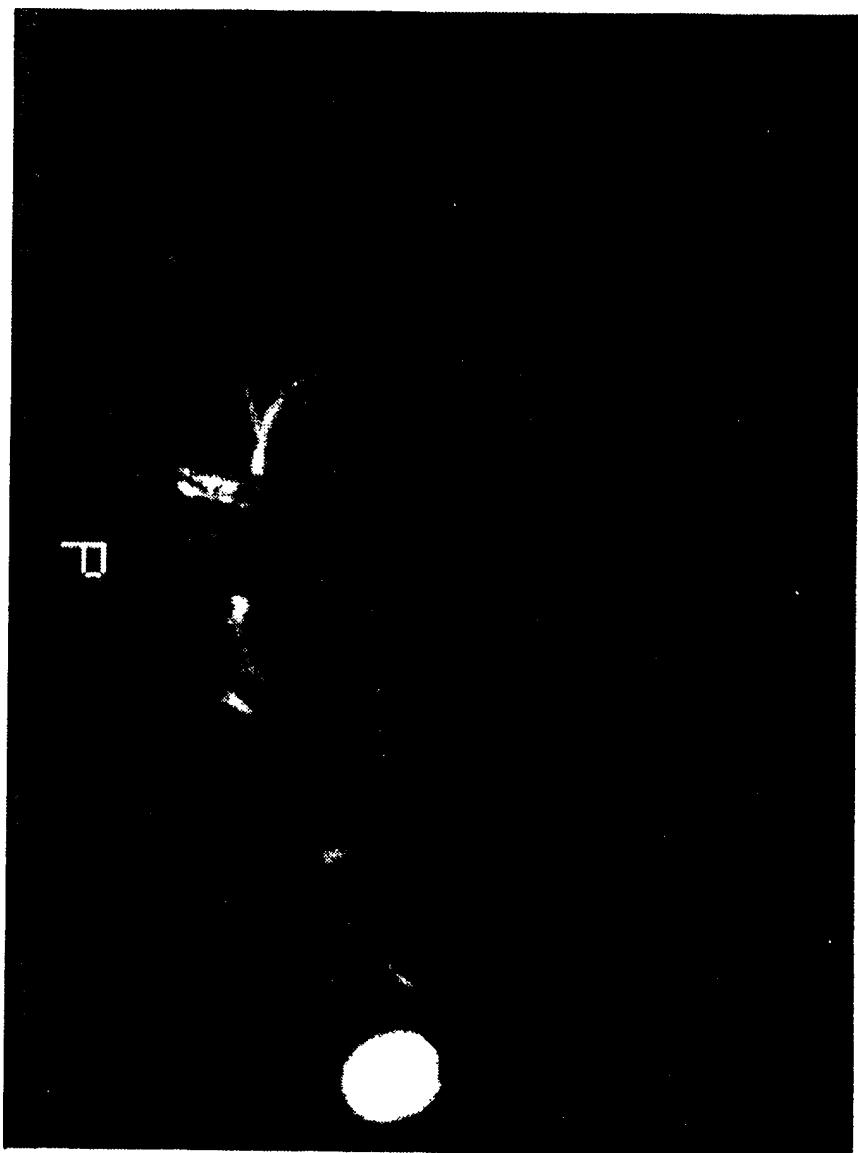


FIG. I

WO 94/08629

PCT/US93/09766



**FIG. 2**



**FIG. 3**

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 93/09766

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 5 A61K49/00

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 5 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	COLLECT. CZECH. CHEM. COMMUN., VOL. 57, PAGES 289-295 February 1992 Makrlik, Emanuel 'Hydration of complexes of some univalent cations with polyethylene glycol ligands in nitrobenzene saturated with water' see the whole document	1-3, 5, 6, 14-17
Y	---	4, 7-13, 18, 19
X	INVEST RADIOL, JAN 1991, VOL. 26, NO. 1, PAGE(S) 50-7, Duewell S et al 'Nonionic polyethylene glycol-ferrioxamine as a renal magnetic resonance contrast agent.' see abstract see page 53	1
Y	---	2-19
	---	-/-

 Further documents are listed in the continuation of box C. Patent family members are listed in annex.

## \* Special categories of cited documents :

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

- \*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- \*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- \*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- \*&\* document member of the same patent family

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Date of the actual completion of the international search

15 February 1994

Date of mailing of the international search report

5. 02.

## Name and mailing address of the ISA

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## Authorized officer

Dullaart, A

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 93/09766

## C(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BIOCHIM BIOPHYS ACTA, 24 MAY 1991, VOL. 1074, NO. 1, PAGE(S) 69-73, Botros HG et al 'Immobilized metal ion affinity partitioning of cells in aqueous two-phase systems: erythrocytes as a model.'	1
Y	* Materials and Methods * ---	2-19
X	INVEST. RADIOL., US, 1990, VOL. 25, SUPPL. 1, PAGE(S) S48, Von Schulthess G.K. et al 'Polyethylene-glycol-ferrioxamine. A new magnetic resonance contrast agent'	1
Y	see the whole document ---	2-19
P,X	J CHROMATOGR, NL, 30 JULY 1993, VOL. 644, NO. 1, PAGE(S) 25-33, Otto A et al 'Recognition and separation of isoenzymes by metal chelates. Immobilized metal ion affinity partitioning of lactate dehydrogenase isoenzymes.'	1
Y	* Materials and Methods * ---	2-19
P,X	RADIOLOGY, US, JUNE 1993, VOL. 187, NO. 3, PAGE(S) 701-6, Bogdanov AA Jr et al 'A new macromolecule as a contrast agent for MR angiography: preparation, properties, and animal studies.'	1-19
Y	* Materials and Methods * ---	
Y	XVTH CONGRESS OF THE EUROPEAN SOCIETY OF CARDIOLOGY, NICE, FRANCE, AUGUST 29-SEPTEMBER 2, 1993; & EUR HEART J, VOL. 14, ABSTR. SUPPL., PAGE(S) 188, ABSTR. NO. 1107, 1993 FRANK H et al 'HIGH RESOLUTION 3-D MR ANGIOGRAPHY OF PULMONARY VESSELS IN AN ANIMAL MODEL' see the whole document ---	1-19
Y	EXP NEUROL, VOL. 70, NO.2, PAGE(S) 452-457, 1980. MOLAK.V. et al 'DYSTROPHIC MOUSE MUSCLES HAVE LEAKY CELL MEMBRANES.' see abstract; figures ---	1-19
Y	EP,A,0 326 226 (MYCOMED AS) 2 August 1989 see claims; examples ---	1-19
10	WO,A,91 15753 (E.C. UNGER) 17 October 1991 see claims; examples ---	1-19
		-/-

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 93/09766

## C(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	NUCLEIC ACIDS RES., 1981, 9, 95-119 Mueller, Werner et al 'Polyethylene glycol derivatives of base and sequence specific DNA ligands: DNA interaction and application for base specific separation of DNA fragments by gel electrophoresis' see page 97 - page 101 ---	1-19
Y	WO,A,85 05554 (AMERSHAM INTERNATIONAL PLC) 19 December 1985 see claims; examples -----	1-19

1

**INTERNATIONAL SEARCH REPORT**

International application No.

**PCT/US 93/09766****Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:  
**REMARK:** Although claims 18 and 19 are directed to a method of treatment of the human or animal body (PCT Rule 39.1 (IV)), the search has been carried out based on the alleged effects of the compound/composition.
2.  Claims Nos.: 1-19 because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:  
**Please see annex.**
3.  Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1.  As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2.  As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3.  As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4.  No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

The additional search fees were accompanied by the applicant's protest.  
 No protest accompanied the payment of additional search fees.

**FURTHER INFORMATION CONTINUED FROM PCT/ISA/210**

**BOX I.2:**

In view of the large number of compounds, which are defined by the general definition of claim 1, and the general formulae of claims 2, 9 and 10, the search had to be restricted for economic reasons. The search was limited to the compounds for which pharmacological data was given and/or the compounds mentioned in the claims, and to the general idea underlying the application (see Guidelines, Part B, Chapter III, paragraph 3.6).

Partially searched claims: 1-19.

**INTERNATIONAL SEARCH REPORT**

Information on patent family members

Int'l. Appl. No

PCT/US 93/09766

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-0326226	02-08-89	AU-A-	3216289	25-08-89
		WO-A-	8906979	10-08-89
		EP-A, B	0396617	14-11-90
		JP-T-	3502338	30-05-91
		US-A-	5208324	04-05-93
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WO-A-9115753	17-10-91	EP-A-	0526503	10-02-93
-----	-----	-----	-----	-----
WO-A-8505554	19-12-85	DE-A-	3585668	23-04-92
		EP-A, B	0183760	11-06-86
		JP-T-	61501571	31-07-86
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